Ultraviolet B-Exposed and Soluble Factor-Pre-Incubated Epidermal Langerhans Cells Fail to Induce Contact Hypersensitivity and Promote DNP-Specific Tolerance

Ruoping Dai and J. Wayne Streilein

The Schepens Eye Research Institute and Department of Dermatology, Harvard Medical School, Boston, Massachusetts, U.S.A.

Acute low-dose ultraviolet B (UVB) radiation impairs the induction of contact hypersensitivity (CH) and induces tolerance in UVB-susceptible strains of mice when dinitrofluorobenzene (DNFB) is applied to an irradiated skin surface. We are interested in learning the cellular and molecular bases for the existence of UVB susceptibility in certain strains of mice. CH was induced by subcutaneous injections into naïve syngeneic C57BL/6 and BALB/c mice of dinitrophenyl (DNP)-derivatized Thy-1$^+$-depleted epidermal cells enriched for Ia$^+$ cells (LC/DNP, 2 × 10^4 cells per mouse). Tolerance was detected by applying 185 μg of DNFB epicutaneously to mice treated 2 wk earlier with a putative tolerating regimen and testing CH expression. We found that LC/DNP obtained from C57BL/6 skin 2 h after UVB irradiation (400 J per m^2) failed to induce CH and induced DNP-specific tolerance instead; by contrast, similar cells obtained from same or even higher dose (400 J per m^2 and 1200 J per m^2) UVB-exposed BALB/c skin induced vigorous CH, and no tolerance was detected. In both C57BL/6 and BALB/c mice, Ia$^+$-depleted EC/DNP neither sensitized naïve syngeneic mice nor induced tolerance. LC/DNP prepared from unirradiated trunk skin of either C57BL/6 or BALB/c mice and pre-incubated *in vitro* for 2 h with *cis*-UCA, TNF-α, or IL-10 failed to induce intense CH; instead, all induced DNP-specific tolerance. Pre-incubation of similar LCs with α-MSH *in vitro* for 2 h also failed to induce CH but did not cause tolerance. Thus, single low-dose UVB irradiation alters the immunogenic and tolerogenic potentials of LCs only in UVB-susceptible mice; by contrast, pre-treatment of LCs with UVB-dependent soluble factors can achieve effects similar to UVB irradiation in both UVB-susceptible and -resistant strains of mice. These findings demonstrate that UVB susceptibility in mice may be determined by the production of UVB-dependent soluble factors within UVB-irradiated skin. ***Key words: antigen presenting cells/sensitization/immunosuppression/UVB susceptibility. J Invest Dermatol 108:721–726, 1997***

Clinical and experimental evidence shows that ultraviolet B (UVB) radiation has deleterious effects on the immune system (Kripke, 1984). Single low-dose (400 J per m^2) UVB radiation significantly impairs the induction of contact hypersensitivity (CH) in UVB-susceptible strains of mice when a low dose (1.5 μg) of dinitrofluorobenzene (DNFB) (referred to as an optimal sensitizing dose) is applied to the irradiated skin surface (Kurimoto and Streilein, 1993). On the basis of supporting experimental evidence, we have proposed that (i) only Langerhans cells (LCs), but not dermal antigen-presenting cells (APCs), participate in CH induction when an optimal sensitizing dose of hapten is applied to the skin surface, and (ii) that a single exposure of skin to UVB radiation perturbs epidermal, but not dermal, APCs. Furthermore, we have recently shown that dinitrophenyl (DNP)-derivatized LCs (LC/DNP), freshly prepared from normal mouse epidermis, induced CH when injected into naïve syngeneic recipients, whereas LC/DNP, prepared from epidermis of UVB-susceptible mice 2 h after UVB (400 J per m^2) exposure, failed to induce CH when the cells were injected into naïve syngeneic recipients (Dai and Streilein, 1995). Thus, these findings suggest that the effects of UVB radiation within the epidermis alter LC functionality and that cells harvested from UVB-exposed epidermis can be studied directly for their susceptibility to the soluble factors generated in skin after UVB radiation.

It is known that the vast majority of UVB light is absorbed within epidermis and that keratinocytes are considered to be the major cutaneous target cells of UVB radiation (Everett *et al*, 1966). Cytokines and/or soluble factors are generated within UVB-exposed skin, and some of these factors possess immunoregulatory properties (Stingl *et al*, 1989). Among these factors, *cis*-urocanic acid (*cis*-UCA), tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10), and α melanocyte stimulating hormone (α-MSH) have been implicated in UVB-induced immune deviation (Rheins *et al*, 1989; Kurimoto and Streilein, 1992; Rivas *et al*, 1992; Enk *et al*, 1993; Schwarz *et al*, 1994). Our laboratory has already reported that

Manuscript received May 20, 1996; revised December 19, 1996; accepted for publication January 6, 1997.

Reprint requests to: Dr. Ruoping Dai, The Schepens Eye Research Institute, Harvard Medical School, 20 Staniford Street, Boston, MA 02114.

Abbreviations: APC, antigen-presenting cell; CH, contact hypersensitivity; LC, Langerhans cell; EC, epidermal cell; α-MSH, α melanocyte-stimulating hormone; UCA, urocanic acid; s.c., subcutaneous(ly).

0022-202X/97/$10.50

intracutaneous injections of *cis*-UCA, mouse recombinant TNF-α, or α-MSH impaired CH induction in a fashion similar to UVB irradiation when hapten was applied to treated skin (Yoshikawa and Streilein, 1990; Shimizu and Streilein, 1994). Other laboratories have reported that systemic administration of *cis*-UCA or murine recombinant IL-10 significantly suppressed contact or delayed type hypersensitivity (Norval *et al*, 1989; Schwarz *et al*, 1994). In addition, it has been documented that incubation of APCs with these factors *in vitro* robs them of accessory functions and prevents upregulation of co-stimulatory molecules (Fiorentino *et al*, 1991; Ding and Shevach, 1992; Macatonia *et al*, 1993). We propose that the soluble factors generated in UVB-exposed skin act directly on epidermal LCs, thereby impairing the cells' ability to induce CH.

In this study, we have tested the capacities of hapten-derivatized LCs prepared from UVB-exposed skin or LCs from normal skin, pre-incubated with UVB-dependent factors *in vitro*, to sensitize naïve syngeneic mice and/or to induce tolerance. We report that LCs obtained from UVB-exposed skin of UVB-susceptible mice, but not UVB-resistant mice, failed to induce CH and induced tolerance instead. Similar results were achieved by subcutaneous (s.c.) injections into naïve syngeneic mice of C57BL/6 and BALB/c LCs pre-incubated with *cis*-UCA, TNF-α, or IL-10. Pre-incubation of LCs with α-MSH impaired CH induction but failed to induce tolerance.

MATERIALS AND METHODS

Mice C57BL/6 and BALB/c female mice, 8–12 wk old, were purchased from Taconic (Germantown, NY) and maintained in our domestic animal facilities.

Reagents Recombinant murine TNF-α was purchased from Genzyme (Cambridge, MA). α-MSH was purchased from Peninsula Laboratories (Belmont, CA). DNFB, oxazolone, and *trans*-urocanic acid (*trans*-UCA) were purchased from Sigma (St. Louis, MO). Anti-mouse Ia^d and anti-mouse Thy-1 monoclonal antibodies were purchased from Becton Dickinson (Bedford, MA). Anti-mouse Ia^b monoclonal antibody was purchased from PharMingen (San Diego, CA).

Preparation of *cis*-Urocanic Acid *trans*-UCA was dissolved in dimethyl sulfoxide at 40 mg per ml and 37°C for 30 min. The solution was subsequently diluted 1:10 in sterile phosphate-buffered saline, as described by Howie *et al*, 1986. A thinly spreading solution was irradiated under four FS-20 fluorescent lamps with a tube-to-target distance of 46 cm for 8 h. By analysis via high performance liquid chromatography, approximately 60% of the irradiated UCA was in the *cis*-isomer.

UVB Radiation Dry shaved abdominal skin of C57BL/6 and BALB/c mice was exposed to UVB from a bank of four fluorescent lamps with tube-to-target distance of 46 cm as described (Toews *et al*, 1980). These tubes have a broad emission spectrum (250–400 nm) and high output primarily in the UVB range (290–320 nm) as measured by an IL 700 radiometer with an SEE 240 UVB photodetector (Medko Medical Instrumentarium, International Light, Newburyport, MA). These lamps delivered an average flux of 1.4 J per m^2. Mice were exposed to a single dose of UVB (400 J per m^2 or 1200 J per m^2) on the shaved abdominal skin surface. Irradiated skin was excised 2 h after UVB exposure and rendered into single-cell suspensions.

Preparation of UVB-Exposed Epidermal Cells (ECs) LC-enriched ECs were prepared as described (Dai *et al*, 1993). Briefly, dry shaved abdominal skin from UVB-exposed mice was removed and floated (epidermis side up) on phosphate-buffered saline containing 0.25% trypsin at 37°C in 5% CO_2 for 1 h. The epidermis was then separated from dermis with fine forceps and incubated in 0.25% trypsin plus 0.5 mg DNase per ml for an additional 10 min. The epidermis was disaggregated by using a 10-ml syringe, and recovered cells were filtered through 100-μm Nitex mesh (Tetko, Elmsford, NY). To enrich for Ia^+ LCs, the suspension was layered on an equal volume of Accu-prep Lymphocyte (Nycomed Pharma AS, Oslo, Norway), and centrifuged at 1600 rpm for 20 min. The recovered interface cells contained 10–15% Ia^+ LCs (referred to as LCs). To deplete Thy-1^+ dendritic epidermal T cells (DETCs), LC-enriched ECs were further incubated with anti-mouse Thy-1 antibody plus complement. To deplete Ia^+ cells from cell suspensions, ECs were treated with anti-mouse Ia^d (BALB/c) or Ia^b (C57BL/6) antibody plus complement.

Pre-Incubation of LCs with UVB-Dependent Factors *In Vitro* LCs prepared from normal unirradiated skin of C57BL/6 and BALB/c mice were incubated with each of the following factors *in vitro*, at 37°C, in 5% CO_2, for 2 h: *cis*-UCA (100 μg per ml), TNF-α (10^5 units per ml), IL-10 (16 ng per ml), or α-MSH (1 ng per ml). Reagents were diluted to desired concentrations in RPMI 1640 medium with 10% fetal bovine serum. Control cells were treated with medium alone under identical conditions. After pre-incubation, LCs were washed and subjected to derivatization with DNFB *in vitro*.

***In Vitro* Derivatization of ECs** EC suspensions enriched or depleted of Ia^+ cells were washed three times and resuspended at 10^6 cells per ml in serum-free RPMI medium 1640. Ten microliters of 0.01% (0.8 mM) DNFB in acetone were added to 1 ml of LC or EC suspension. The mixture was incubated in the dark at room temperature for 30 min with stirring at 10-min intervals. After three washes in fresh medium, the cells were designated as LC/DNP or EC/DNP. Control cells were treated with acetone under similar conditions.

Injections (s.c.) of Hapten-Derivatized ECs DNP-derivatized LCs or Ia^- ECs were adjusted to 2×10^5 cells per ml in RPMI medium 1640 with 10% fetal bovine serum. One hundred microliters of these cells were injected s.c. into hind footpads of naïve syngeneic recipients. Each group in each experiment contained five mice. Each experiment was repeated at least twice with similar results.

Assay for CH Five days after s.c. injections of UVB-exposed or factor-treated LC/DNP, the ear pinnae of treated mice were challenged with 20 μl of 0.1% DNFB (30 μg). Twenty-four hour ear-swelling responses were measured with an engineer's micrometer (Mitutoyo, Osaka, Japan). Positive control mice received skin sensitization with 25 μl of 0.5% DNFB. Negative control mice were similarly challenged with DNFB but had not previously encountered the hapten. Changes in ear thickness at 24 h compared to 0 h (prior to challenge) indicated the extent of CH.

Induction and Assay for Tolerance Two weeks after s.c. injections, dry shaved abdominal skin of injected mice was painted with 25 μl of 0.5% DNFB (185 μg) or 25 μl of 2% oxazolone, whereas uninjected naïve mice were treated with 25 μl of 0.5% DNFB or 2% oxazolone epicutaneously as positive controls. Five days later, ear pinnae were challenged with 20 μl of 0.1% DNFB or 0.2% oxazolone. Net increase in ear thickness at 24 h compared to 0 h indicated the extent of CH. Compared to the increase of ear thickness in positive control mice, significantly lower ear swelling responses were used as the criteria for the induction of tolerance.

Statistical Evaluation of Results The statistical analysis of difference in results was calculated using Student's t test. Differences were considered significant when $p < 0.05$.

RESULTS

UVB-Exposed LCs Fail to Sensitize Naïve Syngeneic Mice and Cause DNP-Specific Tolerance in UVB-Susceptible, but not in UVB-Resistant, Mice We have reported that DNP-derivatized LC-enriched ECs prepared from single-dose (400 J per m^2) UVB-exposed skin of C57BL/6 mice (UVB-susceptible) failed to induce CH when the cells were injected s.c. into naïve syngeneic mice; by contrast, similar cells prepared from UVB-exposed skin of BALB/c mice (UVB-resistant) induced vigorous ear swelling responses (Dai and Streilein, 1995). We first inquired whether hapten-specific tolerance was induced by single-dose UVB-exposed LC/DNP in UVB-susceptible and UVB-resistant strains of mice. If tolerance was not detected in UVB-resistant mice, we wished to determine whether a higher dose of UVB irradiation would promote the induction of tolerance in these mice. In this study, ECs were prepared from UVB-exposed abdominal skin of C57BL/6 and BALB/c mice 2 h after UVB radiation. The ECs were enriched for Ia^+ cells. To exclude the possibility that DETCs may contribute to the induction of tolerance, LC-enriched ECs were further depleted of Thy-1^+ cells by incubating the EC suspension with anti-mouse Thy-1 antibody plus complement. To confirm that tolerance-inducing cells are LCs, some EC suspensions were depleted of Ia^+ cells by incubating the cells with anti-mouse Ia^d or Ia^b antibody plus complement. After derivatization with DNFB *in vitro*, DNP-bearing LCs or ECs (2×10^4 cells in 100 μl per mouse) were injected s.c. into hind footpads of naïve syngeneic recipients. Positive control mice received injections of LC/DNP prepared from skin of unirradiated mice. Five days after s.c. injection, CH was assayed by challenging the ear skin with dilute

Table I. UVB-Exposed LCs Fail to Induce CH and Promote DNP-Specific Tolerance

	Sensitization (First)	Secondary sensitization	Ear Swelling Responses (μm) C57BL/6	BALB/c
I. Induction of CH[a]	None		15 ± 6	10 ± 3
	Epicutaneous DNFB		99 ± 13	85 ± 8
	LC/DNP s.c.		63 ± 7	70 ± 2
	UVB-LC/DNP s.c. (400 J per m^2)		28 ± 9[b]	70 ± 7
	UVB-LC/DNP s.c. (1200 J per m^2)		ND	68 ± 5
II. Induction of DNP-specific tolerance[c]	None	None	20 ± 5	10 ± 3
	None	Epicutaneous DNFB	147 ± 11	73 ± 3
	LC/DNP s.c.	Epicutaneous DNFB	135 ± 14	78 ± 4
	UVB-LC/DNP s.c. (400 J per m^2)	Epicutaneous DNFB	72 ± 10[d]	77 ± 5
	UVB-LC/DNP s.c. (400 J per m^2)	Epicutaneous oxazolone	135 ± 7	96 ± 7
	UVB-LC/DNP s.c. (1200 J per m^2)	Epicutaneous DNFB	ND	111 ± 23
	UVB-LC/DNP s.c. (1200 J per m^2)	Epicutaneous oxazolone	ND	123 ± 6
	None	Epicutaneous DNFB	120 ± 10	103 ± 6

[a] Induction of CH: C57 BL/6 and BALB/c mice received either epicutaneous painting with 25 μl of 0.5% DNFB or s.c. injections of syngeneic LC/DNP prepared from untreated skin or skin 2 h after UVB exposure (2×10^4 cells per mouse). Mice were then ear-challenged with 20 μl of 0.1% DNFB 5 days later, and 24-h ear swelling responses were measured. Net thickness increases of ear skin were referred to as the extent of CH.

[b] Responses significantly less than positive control (epicutaneous DNFB) ($p < 0.05$).

[c] Induction of DNP-specific tolerance. C57BL/6 and BALB/c mice that previously received s.c. injections of UVB-exposed LC/DNP were resensitized by epicutaneous painting with 25 μl of 0.5% DNFB or 2% oxazolone 2 wk after injections. As positive controls, naive mice were sensitized epicutaneously with 25 μl of 0.5% DNFB or 2% oxazolone. Upon challenge with same haptens 5 d later, ear swelling repsonses were measured as described above.

[d] Responses significantly less than positive (epicutaneous DNFB) ($p < 0.001$).

DNFB. The results of representative experiments are presented in **Table I**. LC/DNP from unirradiated skin of C57BL/6 and BALB/c mice induced intense CH in naïve syngeneic mice. LC/DNP prepared from UVB-exposed skin (400 J per m^2) of C57BL/6 mice, however, failed to induce CH, whereas similar cells from UVB-exposed skin (400 J per m^2 and 1200 J per m^2) of BALB/c mice elicited intense ear swelling responses. These results further confirm our previous findings. To test whether hapten-specific tolerance was also induced in these injected mice, 14 d after injections, previously injected C57BL/6 and BALB/c mice received a secondary sensitization by an epicutaneous application of 185 μg of DNFB. As a hapten-specific control, some injected mice received epicutaneous application of the unrelated hapten, oxazolone. Five days later, the ears were challenged with dilute DNFB or oxazolone. Twenty-four hour ear swelling responses were assessed for the extent of CH, which is used as an indication of tolerance. The results presented in **Table I** showed that C57BL/6 mice first treated with UVB-exposed LC/DNP displayed significantly less ear swelling in response to DNFB, but not to oxazolone, than untreated controls, suggesting that DNP-specific tolerance was induced. BALB/c mice exposed to the same or even higher doses of UVB developed CH as intense as positive controls, suggesting that tolerance was not induced in these mice. In both C57BL/6 and BALB/c mice, Ia^+-depleted EC/DNP neither sensitized naïve syngeneic mice nor induced tolerance (data not shown). This result confirms that a tolerance-inducing signal within ECs from UVB-exposed skin is associated with LCs.

LCs Pre-Incubated with *cis*-UCA, TNF-α, IL-10, or α-MSH Fail to Induce CH but Induce Tolerance in Naïve Syngeneic UVB-Susceptible and -Resistant Mice We and other laboratories have shown that intracutaneous administration of *cis*-UCA and TNF-α impaired CH induction and induced tolerance when DNFB was applied to the treated skin surface. Recently, this laboratory (Niizeki and Streilein, submitted) has found that intracutaneous injection of mouse recombinant IL-10 also induced tolerance, although it did not impair CH induction. *cis*-UCA, TNF-α, and IL-10 are soluble factors generated within UVB-exposed skin and are candidates to mediate the deleterious effects of UVB radiation on cutaneous immunity. To determine whether these factors can directly alter the immunogenic and/or tolerogenic potentials of hapten-bearing LCs in both strains of mice, we prepared LCs from normal skin of C57BL/6 and BALB/c mice were pre-incubated with one of these factors *in vitro* for 2 h and then derivatized with DNFB *in vitro*. The cells were injected s.c. into naïve syngeneic recipients, and CH and tolerance were assayed as described previously. The results of representative experiments **(Figs 1-3)** show that LCs pre-incubated with each of these factors

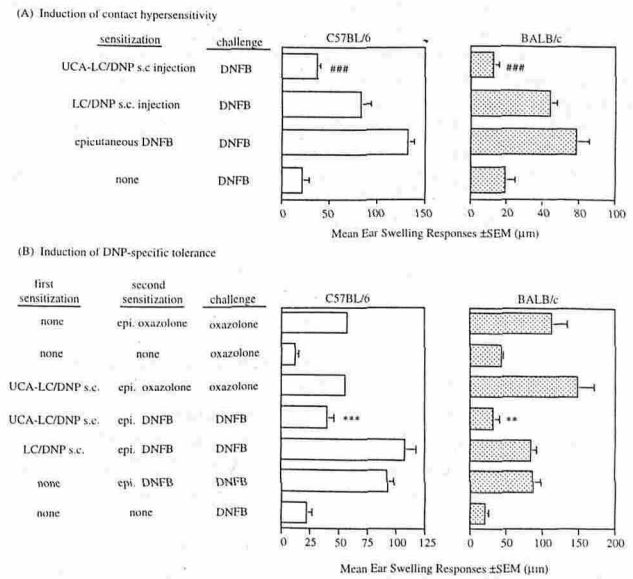

Figure 1. LCs pre-treated with *cis*-UCA fail to induce CH and cause tolerance. *(A)* Induction of CH in C57BL/6 and BALB/c mice that received s.c. injections of DNP-haptenated ECs enriched for LCs pre-treated with *cis*-UCA (UCA-LC/DNP). LCs prepared from normal skin and derivatized with DNFB *in vitro* were injected into hind footpads of naïve syngeneic mice (2×10^4 cells in 100 μl per mouse). Positive control mice received epicutaneous application of 25 μl of 0.5% DNFB. Five days later, left ear pinnae were challenged with 20 μl of 0.1% DNFB, and 24-h ear swelling responses were measured. Net increases in ear skin thickness at 24 h are presented. *Error bars*, SEM. ###, Ear swelling responses significantly less than positive control ($p < 0.001$). *(B)* Induction of DNP-specific tolerance in C57BL/6 and BALB/c mice injected with LC/DNP treated with *cis*-UCA 2 wk previously. Dry shaved abdominal skin of injected mice was painted with 25 μl of 0.5% DNFB or 2.0% oxazolone. Five days later, right ear pinnae were challenged with 20 μl of 0.1% DNFB or 0.2% oxazolone, and 24-h ear swelling responses were measured. Ear swelling responses significantly less than positive control and indicated: ***, $p < 0.001$; **, $p < 0.0001$. *Error bars*, SEM ($n = 5$).

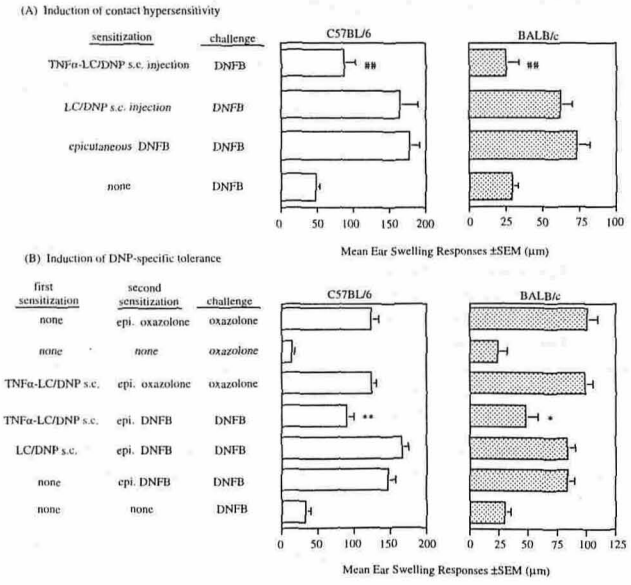

Figure 2. LCs pre-treated with TNF-α fail to induce CH and cause tolerance. *(A)* Induction of CH in C57BL/6 and BALB/c mice that received s.c. injections of DNP-haptenated ECs enriched for LCs pre-treated with TNF-α (TNF-α-LC/DNP). LCs, prepared from normal skin and derivatized with DNFB *in vitro*, were injected into hind footpads of naïve syngeneic mice (2×10^4 cells in 100 μl per mouse). Positive control mice received 25 μl of 0.5% DNFB epicutaneously. Five days later, left ear pinnae were challenged with 20 μl of 0.1% DNFB, and 24-h ear swelling responses were measured. Net increases in ear skin thickness at 24 h are presented. *Error bars*, SEM. ##, Ear swelling responses significantly less than positive control ($p < 0.001$). *(B)* Induction of DNP-specific tolerance in C57BL/6 and BALB/c mice injected with LC/DNP treated with TNF-α 2 wk previously. Dry shaved abdominal skin of injected mice was painted with 25 μl of 0.5% DNFB or 2.0% oxazolone. Five days later, right ear pinnae were challenged with 20 μl of 0.1% DNFB or 0.2% oxazolone, and 24-h ear swelling responses were measured. Ear swelling responses significantly less than positive control are indicated: *, $p < 0.05$; **, $p < 0.001$. *Error bars*, SEM (n = 5).

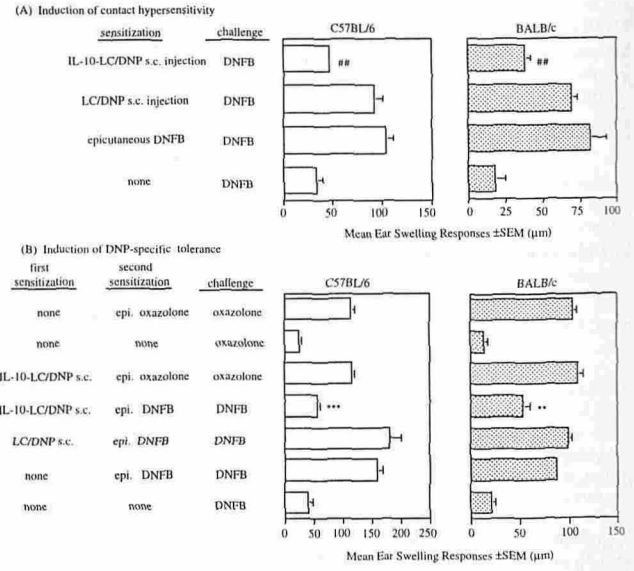

Figure 3. LCs pre-treated with IL-10 fail to induce CH and cause tolerance. *(A)* Induction of CH in C57BL/6 and BALB/c mice that received s.c. injections of DNP-haptenated ECs enriched for LCs pre-treated with IL-10 (IL-10-LC/DNP). LCs, prepared from normal skin and derivatized with DNFB *in vitro*, were injected into hind footpads of naïve syngeneic mice (2×10^4 cells in 100 μl per mouse). Positive control mice received 25 μl of 0.5% DNFB epicutaneously. Five days later, left ear pinnae were challenged with 20 μl of 0.1% DNFB, and 24-h ear swelling responses were measured. Net increases in ear skin thickness at 24 h are presented. *Error bars*, SEM. ##, Ear swelling responses significantly less than positive control ($p < 0.001$). *(B)* Induction of DNP-specific tolerance in C57BL/6 and BALB/c mice injected with LC/DNP treated with IL-10 2 wk previously. Dry shaved abdominal skin of injected mice was painted with 25 μl of 0.5% DNFB or 2.0% oxazolone. Five days later, right ear pinnae were challenged with 20 μl of 0.1% DNFB or 0.2% oxazolone, and 24-h ear swelling responses were measured. Ear swelling responses significantly less than positive control are indicated: **, $p < 0.001$; ***, $p < 0.0001$. *Error bars*, SEM (n = 5).

lost their capacity to sensitize naïve syngeneic mice, and in each instance, factor-exposed LC/DNP led to the development of tolerance. These results are almost completely comparable to previously reported findings of *in vivo* treatment of skin with the same factors. The only discrepancy concerns IL-10, which, in our protocols, did not impair CH induction *in vivo* but did prevent *in vitro*-exposed LC/DNP from inducing CH.

Circumstantial evidence indicates that the numbers of melanocytes at UVB-exposed skin are increased, and it has been reported that α-MSH activity is increased in the blood of individuals exposed to UVB radiation (Kaidbey *et al*, 1979). We are therefore interested in the immunoregulatory potential of α-MSH, because it displays many activities that antagonize the effects of pro-inflammatory cytokines and because epicutaneous application of α-MSH prior to painting with hapten has been shown to impair CH induction but not to induce tolerance. Accordingly, LCs were treated *in vitro* with α-MSH, then derivatized, and used to induce CH in naïve syngeneic mice. The results presented in **Fig 4** reveal that pre-incubation of LCs with α-MSH significantly impaired their ability to induce CH. Unlike the other UVB-dependent factors mentioned above, however, LCs treated with α-MSH did not induce tolerance in injected mice. Thus, the data indicate that all of the UVB-dependent soluble factors used in our current protocol can alter the immunogenic and/or tolerogenic potentials of hapten-bearing LCs in both UVB-susceptible and resistant strains of mice.

DISCUSSION

The current experiments provide important information concerning potential mechanisms by which UVB radiation interferes with CH induction when an optimal sensitizing dose of hapten (presumably derivatized ECs only) is painted on exposed skin of UVB-susceptible mice (such as C57BL/6). We have previously reported that LCs harvested from epidermis of C57BL/6 mice 2 h after UVB exposure were unable to sensitize naïve syngeneic mice to the hapten in question. This inability contrasted sharply with that of LCs from UVB-exposed skin of UVB-resistant mice, BALB/c, suggesting that UVB radiation may have a *direct* effect on LCs in C57BL/6 mice, rendering them relatively incompetent as APCs. Since after a single dose (400 J per m^2) of UVB radiation, viable LCs remain in the epidermis of both UVB-resistant and UVB-susceptible mice, it is possible that the UVB-dependent environment that surrounds LCs in exposed epidermis can cause functional changes in the LCs of UVB-susceptible mice but not in UVB-resistant mice. UVB radiation alters keratinocytes in a manner that leads to upregulation of numerous genes encoding pro-inflammatory and immunomodulatory cytokines (Luger *et al*, 1990). In support of this view, we have shown previously that intracutaneous injections of *cis*-UCA or subinflammatory amounts of TNF-α impair CH induction in both UVB-susceptible and UVB-resistant mice and that these effects are largely reversed by neutralizing anti-TNF-α antibodies (Yoshikawa and Streilein, 1990; Kurimoto and Streilein, 1992). Our present findings confirm that *cis*-UCA, TNF-α, IL-10, and α-MSH act directly on epidermal LCs *in vitro*,

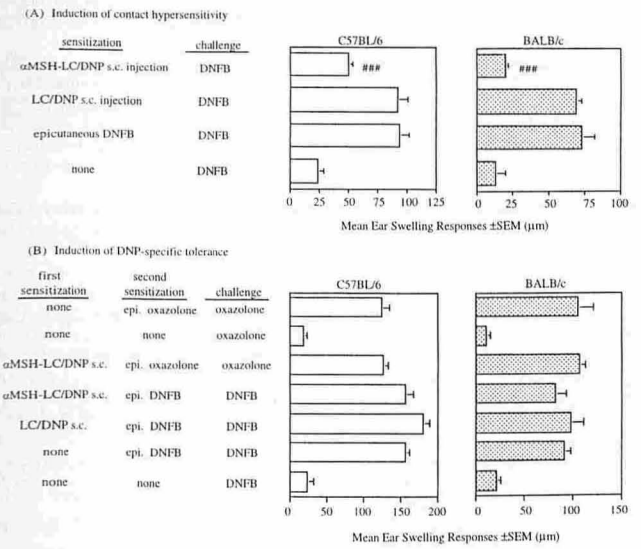

Figure 4. LCs pre-treated with α-MSH fail to induce CH induction but do not cause tolerance. *(A)* Induction of CH in C57BL/6 and BALB/c mice that received s.c. injections of DNP-haptenated ECs enriched for LCs pre-treated with α-MSH (αMSH-LC/DNP). LCs, prepared from normal skin and derivatized with DNFB *in vitro*, were injected into hind footpads of naïve syngeneic mice (2×10^4 cells in 100 μl per mouse). Positive control mice received 25 μl of 0.5% DNFB epicutaneously. Five days later, left ear pinnae were challenged with 20 μl of 0.1% DNFB, and 24-h ear swelling responses were measured. Net increases in ear skin thickness at 24 h are presented. *Error bars*, SEM. ###, Ear swelling responses significantly less than positive control ($p < 0.001$). *(B)* Induction of DNP-specific tolerance in C57BL/6 and BALB/c mice injected with LC/DNP treated with α-MSH 2 wk previously. Dry shaved abdominal skin of injected mice was painted with 25 μl of 0.5% DNFB or 2.0% oxazolone. Five days later, right ear pinnae were challenged with 20 μl of 0.1% DNFB or 0.2% oxazolone, and 24-h ear swelling responses were measured. *Error bars*, SEM ($n = 5$).

such that hapten-bearing LCs treated in this manner lose their ability to induce CH. Alternatively, these soluble factors may act on keratinocytes, and factors released from keratinocytes may rob LCs of their functional potential to induce CH. Moreover, we believe that the experimental approach we have adopted may enable us to identify the features of LCs that are altered directly or indirectly by UVB radiation.

Failed CH induction after acute low-dose UVB radiation is not the only immunologic outcome. In UVB-susceptible mice, hapten-specific tolerance is also induced (Toews *et al*, 1980). The role of ECs in promoting tolerance of this type has been considered previously, and both epidermal LCs and Thy-1+ DETCs have been implicated (Sullivan *et al*, 1986). There is little question that hapten-derivatized DETCs can induce tolerance. What has been open to question is whether DETCs or LCs from UVB-exposed epidermis actually participate in the tolerance that emerges when hapten is painted on UVB-treated skin. At high doses of UVB radiation, including our four-dose protocol, virtually no LCs remain in the epidermis, and DETCs are markedly reduced in density (Toews *et al*, 1980; Aberer *et al*, 1986). In this context, it is difficult to imagine how epidermal LCs could participate in UVB-dependent tolerance. After a single low dose of UVB radiation (1×400 mJ per cm^2), however, an optimal sensitizing dose of hapten not only fails to induce CH but also fails to induce tolerance (Kurimoto and Streilein, 1993). This circumstantial evidence militates against a role for epidermal LCs in tolerance induction after either high or low doses of UVB radiation. Nonetheless, our experiments indicate that hapten-derivatized LCs obtained from epidermis 2 h after a one-dose UVB treatment induced tolerance when injected into naïve syngeneic recipients. In fact, *in vitro* treatment of LC-enriched cell suspensions with *cis*-UCA, TNF-α, or IL-10 also imparted to the cells tolerance-promoting activity. These findings resemble those reported by Cruz *et al* (1989), who demonstrated that Ia^+ ECs exposed to UVB radiation *in vitro* induced tolerance in recipient mice. Our conclusion is that LCs, perturbed directly by UVB radiation or by factors released into the UVB-exposed epidermal compartment, can induce tolerance. For reasons already stated, however, we view with circumspection the proposal that LCs actually participate in the tolerance that is achieved *in vivo* after any dose of UVB radiation. Instead, we believe that either DETCs provide the tolerance-conferring stimulus from UVB-treated skin or other cells present within the dermis provide this function. To that end, Kurimoto and Streilein (1994) reported recently that after a four-dose UVB exposure, the dermis contains nonphagocytic cells with the capacity to induce hapten-specific tolerance. Whether the relevant cells are dermal LCs or some other bone marrow-derived cell recruited to the dermis by UVB radiation damage is the subject of our continuing inquiry.

Our findings provide important information helping us to understand the mechanisms of UVB susceptibility. Because LCs prepared from UVB-exposed epidermis of UVB-susceptible mice failed to immunize naïve syngeneic mice and promoted DNP-specific tolerance instead but similar cells from UVB-resistant mice retained their capacity to induce CH but did not cause tolerance, it seems likely that the differential effects of UVB radiation on LC's functional properties in different strains of mice are determined by quantitatively different amounts of soluble factors released into UVB-exposed epidermis where LCs reside. More interestingly, effects similar to those induced by UVB radiation *in vivo* can be achieved by pre-incubating LCs from normal skin of both UVB-susceptible and -resistant mice with UVB-dependent soluble factors. Vincek *et al* (1993) have shown that polymorphism exists at the *Tnfα* locus of numerous inbred strains expressing the UVB-susceptible and -resistant traits. We postulate that the *Tnfα* allele of UVB-resistant mice imparts poor transcriptional efficiency at *Tnfα* compared to the alleles of UVB-susceptible mice and suggest that quantitative differences in TNF-α produced intracutaneously in response to UVB radiation may account for the phenotyic traits of UVB-susceptible and -resistant. Yoshikawa and Streilein (1990) have reported that C3H/HeN (UVB-susceptible) and C3H/HeJ (UVB-resistant) are differentially susceptible to the deleterious effects of intradermally injection of TNF-α on the induction of CH and that the difference is related to the allelic differences at *Lps*. Dose-dependent responses of LCs to UVB-related factors should be further explored to determine whether LCs display different sensitivity to UVB effects.

This work was supported by U.S. Public Health Service Grant AI122072.

REFERENCES

Aberer W, Romani N, Eble A, Stingl G: Effects of physiochemical agents on murine epidermal Langerhans cells and Thy-1-positive dendritic cells. *J Immunol* 136: 1210–1216, 1986

Cruz PD Jr, Nixon-Fulton J, Tigelaar RE, Bergstresser PR: Disparate effects of *in vitro* low-dose UVB irradiation on intravenous immunization with purified epidermal subpopulations for the induction of contact hypersensitivity. *J Invest Dermatol* 92:160–165, 1989

Dai R, Grammer SF, Streilein JW: Fresh and cultured Langerhans cells display differential capacities to activate hapten-specific T cells. *J Immunol* 150:59–66, 1993

Dai R, Streilein JW: Sensitizing capacity of Langerhans cells obtained from ultraviolet-B-exposed murine skin. *Immunology* 86:661–667, 1995

Ding L, Shevach EM: IL-10 inhibits mitogen-induced T cell proliferation by selectively inhibiting macrophage costimulatory function. *J Immunol* 148:3133–3139, 1992

Enk AH, Angeloni VL, Udey MC, Katz SI: Inhibition of Langerhans cells antigen-presenting function by IL-10. A role for IL-10 in induction of tolerance. *J Immunol* 151:2390–2398, 1993

Everett M, Yeargers E, Sayre R, Olson R: Penetration of epidermis by ultraviolet rays. *Photochem Photobiol* 5:533–542, 1966

Fiorentino DF, Zlotnik A, Vieira P, Mosmann TR, Howard M, Moore KW, O'Garra A: IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *J Immunol* 146:3444–3451, 1991

Howie A, Norval M, Maingan J: *Exposure to low dose ultraviolet radiation suppresses* delayed- type hypersensitivity to herpes simple virus in mice. *J Invest Dermatol* 86:125–128, 1986

Kaidbey KJ, Agin PP, Sayre RM, Kligman AM: *Photoprotection by melanin—a* comparison of black and caucasian skin. *J Am Acad Dermatol* 1:249–260, 1979

Kripke ML: Immunological unresponsiveness induced by UV radiation. *Immunol Rev* 80:87–102, 1984

Kurimoto I, Arana M, Streilein JW: Role of dermal cells from normal and ultraviolet B-damaged skin in induction of contact hypersensitivity and tolerance. *J Immunol* 152:3317–3323, 1994

Kurimoto I, Streilein JW: *cis*-Urocanic acid suppression of contact hypersensitivity induction is mediated via tumor necrosis factor-α. *J Immunol* 148:3072–3078, 1992

Kurimoto I, Streilein JW: *Studies of contact* hypersensitivity in mice with optimal sensitizing doses of hapten. *J Invest Dermatol* 101:132–136, 1993

Luger TA, Schwarz T: Epidermal cell-derived cytokines. In: Bos JD (ed.). *Skin Immune System*. CRC, Boca Raton, FL, 1990, p 257

Macatonia SE, Doherty TM, Knight SC, O'Garra A: Differential effect of IL-10 on dendritic cell-induced T cell proliferation and IFN-gamma production. *J Immunol* 150:3755–3765, 1993

Norval M, Simpson TJ, Ross JA: Urocanic acid and immunosuppression. *Photochem Photobiol* 50:267–275, 1989

Rheins LA, Cotleur AL, Kleier RS, Hoppenjans WB, Sauder DN, Nordlund JJ: Alpha *melanocyte* stimulating hormone modulates contact hypersensitivity responsiveness in C57BL/6 mice. *J Invest Dermatol* 93:511–517, 1989

Rivas JM, Ullrich SE: Systemic suppression of delayed-type hypersensitivity by supernatant from UV-irradiated keratinocytes. An essential role for keratinocyte-derived IL-10. *J Immunol* 149:3865–3871, 1992

Schwarz A, Grabbe S, Riemann H, Aragane Y, Simon M, Manon S, Andrade S, Luger TA, Zlotnik A, Schwarz T: *In vivo* effects of interleukin-10 on contact hypersensitivity and delayed-type hypersensitivity reactions. *J Invest Dermatol* 103:211–216, 1994

Shimizu T, Streilein JW: Influence of alpha-melanocyte stimulating hormone on induction of contact hypersensitivity and tolerance. *J Dermatol Sci* 8:187–193, 1994

Stingl G, Tschachler E, Groh V, Wolff K: The Immune Function of Epidermal Cells. In: Norris D (ed.). *Immune Mechanisms in Cutaneous Disease*. Marcel Dekker, New York, 1989, p 4

Sullivan S, Bergstresser PR, Streilein JW: Induction and regulation of contact hypersensitivity by resident bone-marrow derived, dendritic epidermal cells: Langerhans cells and Thy-1+ dendritic cells. *J Immunol* 137:2460–2467, 1986

Toews G, Bergstresser PR, Streilein JW: Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. *J Immunol* 124:445–453, 1980

Vincek V, Kurimoto I, Medema JP, Prieto E and Streilein JW: Tumor necrosis factor a polymorphism correlates with deleterious effects of ultraviolet B light on cutaneous immunity. *Cancer Res* 53728–732, 1993

Yoshikawa T, Streilein JW: Genetic basis of the effects of ultraviolet light B on cutaneous immunity. Evidence that polymorphism at the *Tnfα* and *Lps* loci governs susceptibility. *Immunogenetics* 32:398–405, 1990